Plenary lectures

Possible therapeutic use an endogenous amine from tetrahydroisoquinoline group

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1,2,3,4-tetrahydroisoquinoline (TIQ) and specially its close methyl derivative, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) displays neuroprotective and antiaddictive properties unlike several other tetrahydroisoquinolines with rather neurotoxic mechanism of action in the brain (e.g. salsolinol, 1-benzyl-1,2,3,4-tetrahydroisoquinoline, 1BnTIQ). To elucidate this action we compared the effect of 1MeTIQ with 1BnTIQ on locomotor activity and dopamine metabolism and its catabolism: N-oxidative and O-methylation catabolic pathways. The experiments have shown that both compounds produce different behavioral and neurochemical effects on the dopaminergic system of the rats after a single administration as well as after chronic treatment. 1BnTIQ depressed the locomotor activity of rats and produces a dramatic fall of dopamine level but a distinct increase in DOPAC and HVA concentrations in the striatum and nucleus accumbens. Interestingly, the effects of chronic administration of 1BnTIQ are different: the effects in the nerve ending containing areas are smaller, but the depression of dopamine level in the substantia nigra is appreciable and comparable with that in the striatum and nucleus accumbens. This pattern of changes suggests that during a chronic administration of 1BnTIQ a tolerance to its dopamine-releasing effect develops, while the impairment of dopamine synthesis ensues. These results may suggest that 1BnTIQ as endogenous substance which strongly potentiates MAO-dependent dopamine oxidation and impairs dopamine storage inside the nerve endings may be one of the factors responsible for idiopathic Parkinson's disease. In contrast to 1BnTIQ, 1-methyl-derivative of endogenous tetrahydroisoquinoline amine, 1MeTIQ expressed a neuroprotective activity in many behavioral and neurochemical experiments. 1MeTIQ did not change the behavior and locomotor activity of naive rat, however clearly affects dopamine catabolic pathways in many investigated brain structures (substantia nigra, VTA, striatum, nucleus accumbens). 1MeTIQ opposite to neurotoxic 1BnTIQ inhibits the dopamine MAOB dependent N-oxidation, and accelerates at least twice the COMT-dependent O-methylation; however the rate of dopamine metabolism was not change in any investigated structures. Such effect on dopamine catabolism produced by 1MeTIQ may reduce the generation of free radicals accompanying this process, and can favour of a neuroprotection, and seems to be interesting in the context of potential clinical application. *In vitro* and *in vivo* studies have shown in details the mechanism of neuroprotection produced by 1MeTIQ in rodents brain which is closely connected with free radicals scavenging properties and inhibition of glutamate- and kainate-induced excitotoxicity [Antkiewicz-Michaluk et al., J Neurochemistry, 2006]. Interestingly, 1MeTIQ expressed also considerable potential as a drug for combating substance abuse disease through the attenuation of craving and abstinent syndrome in cocaine and morphine-dependent rats [Antkiewicz-Michaluk et al., J Neural Transmission, 2007; Wąsik et al., J Physiol Pharmacol, 2007]. In summary, the results strongly support the view that 1MeTIQ has a fundamental capability to be used in clinic as a drug with neuroprotective and antiaddictive properties.

Autophagy in brain ischemia – potential target for neuroprotective strategies?

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Brain ischemia is the third leading cause of death in economically developed countries. Currently the only pharmacological therapy available clinically is rt-PA admission, however its effectiveness is low due to very short therapeutic window. Thus causes a great interest in developing alternative forms of therapy that prevent ischemic cascade and minimize the subsequent neurodegeneration. Recent research revealed that pharmacological modulation of autophagy might be a potential target of new neuroprotective strategies. Autophagy is an intracellular bulk degradation system, that allows elimination of damaged, used and dead cellular constituents, therefore it produces substrates for energy generation and serves to support homeostasis. Deregulation of autophagy contributes to several neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's. Furthermore up-regulation of autophagy is observed during periods of nutrients depletion. Because of autophagy catabolic function its cytoprotective role during stress conditions is now generally accepted. On the other hand over-activated autophagy might be detrimental and even lead to cell death. Moreover cross-talk between autophagy and apoptosis that can lead to programmed cell death has been affirmed. Recent studies indicated unequivocal activation of autophagy during cerebral ischemia, yet role of this activation sparked controversy, indicating necessity to intensify research in this field.

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"Oncological" bevacizumab *vs*. "ophthalmological" ranibizumab: are they both for exudative-neovascular AMD?

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Bevacizumab and ranibizumab are closely related humanized (from mouse) monoclonal antibodies targeting all isoforms of the vascular endothelial growth factor (VEGF)-A family. Bevacizumab is a 149 kDa full-length antibody, whereas ranibizumab is largely the same antibody pruned down to its active fragment of 48 kDa and enhanced for greater anti-VEGF potency. Ranibizumab shows thus 1/3 the size of the parent molecule. Both antibodies were invented and manufactured until now by Genentech (San Francisco, CA, USA). Since VEGF is a major proangiogenic factor in physiology, and also in diseased conditions – having a role in promoting pathological neovascularization, both bevacizumab and ranibizumab have found clinical application as anti-angiogenesis agents.

The former drug named Avastin (Genentech/Roche) was officially approved for the treatment – in combination with standard chemotherapy – of metastatic colorectal cancer (2004, FDA). Later on, official indications for Avastin have expanded to other oncological diseases, such as non-squamous-non-small-cell lung cancer, metastatic renal cell cancer, metastatic breast cancer, and glioblastoma multiforme. Avastin is supplied in single-use glass vials containing sterile solution of bevacizumab: 100 mg/4 mL and 400 mg/16 mL for intravenous infusion (*iv*).

The latter drug named Lucentis (Genentech/Novartis) was officially approved as an agent specially prepared for the treatment of ophthalmological disease, ie. neovascular (wet or exudative) form of age-related macular degeneration – AMD (2006/2007, FDA/EMEA). Neovascular AMD is the leading cause of adult visual impairment and irreversible blindness; it is characterized by the formation of choroidal neovascularization (CNV), which extends beneath (subretinally) or into the retina in the macular region. Lucentis is commercially distributed in vials containing 0.2 mL of sterile solution with 2 mg ranibizumab for one intravitreal (*ivt*) application; the recommended dose is 0.5 mg/0.05 mL per injection.

Despite that bevacizumab was unlicensed for ocular neovascularization, it had been successfully used, before Lucentis approval, as an *off-label* treatment in patients with wet form AMD. The drug (Avastin) was firstly applied *via* peripheral route, and then administered directly into the eye (*ivt*) in a dose of 1.25 mg, ie. 0.05 mL of the original *iv* solution. The results of these first "ophthalmological" trials with bevacizumab were published in 2005. Since that time, Avastin *ivt* was repeatedly, and in fact continuously until now, used in AMD patients, in spite of the fact that the eye-directed Lucentis was already present on the pharmaceutical market, being all the time under intensive promotion.

Interestingly, according to the U.S. Medicare data for 2009, 71% of patients with neovascular AMD received *off-label* Avastin *ivt*, 26% – Lucentis *ivt*, and 3% both drugs. Concerning the period 2004–2008, the Medicare data, embracing nearly half a million of American AMD patients treated with the VEGF-antibodies, were similar to those cited for 2009. A roughly similar statistics concerning the therapeutic usage of either Avastin or Lucentis for neovascular AMD can be seen in other countries throughout the world, including the author's country. Generally speaking, the use of "oncological" bevacizumab for neovascular ocular problems largely exceeded in the past, and currently still exceeds that of "ophthalmological" ranibizumab. The reason for such a curious situation, where an unlicensed agent is decisevly more popular than licensed drug, finds its explanation in costs rather than clinical efficacy. It should be stressed that one intravitreal dose of Avastin costs approximately US$ 40–50 whereas that of Lucentis – approxoimately US$ 2,000. Monthly injections of 0.5 mg Lucentis cost more than US$ 23,000 per patient annually, which distinctly exceeds the annual cost of the Avastin *ivt* therapy. Already wide clinical experience with the use of both monoclonal VEGF-antibodies against neovascular AMD does not point out any major difference between them. The two agents seem to represent similar, if not the same pharmacological and clinical profiles.

However, according to some opinions, a larger molecular mass for bevacizumab may limit its penetration through ocular tissues, thus contributing to possibly poorer or delayed therapeutic output (compared to Lucentis), yet such an opinion does not find support in published experimental data. Being a larger molecule, bevacizumab may remain in the eye longer than smaller in size ranibizumab – this in fact might have two consequences – negative (eg. slower elimination of the drug, which could increase the risk of unwanted effects) and positive (eg. the need for less frequent injections; usually, the routine treatment includes at least three monthly injections of the drug, and then the therapy is continued as needed, with further monthly treatments). Some ophthalmologists rise another possible problem with bevacizumab *ivt* – an unproven safety, compared to thoroughly tested Lucentis *ivt* that appears to be a drug without any serious side effects. Regarding bevacizumab *ivt*, such an objection theoretically might be valid; however, taking into account thousands of therapeutically successful intraocular injections of Avastin that were made since the first trials in 2004/2005 until now in dozens of countries all over the world, it is hardly to see any superiority of Lucentis over Avastin *ivt* treatment.

In order to firmly justify the notion on therapeutic equivalence of the two agents, and to stop discussions which drug is better, more effective, safer, etc., head-to-head comparison of clinical effectiveness of *ivt* bevacizumab or ranibizumab in neovascular AMD would be of great value. However, the distributors of these agents – Novartis (Lucentis) and Roche (Avastin), as well as the manufacturer – Genentech, are not interested in such studies. Fortunately, such pharmaceutical industry independent studies, inspired and funded by either the official ophthalmological organizations or governmental instituitions, are currently in progress and the results are expected soon; their aim is to investigate not only clinical outputs, such as efficacy, safety, quality of life, variations in treatment modalities, but also costs of the therapy. The up-to-now clinical experience and collected experimental data prompt likely conclusion – clinical equivalence of "oncological" Avastin with "ophthalmological" Lucentis. In the author's opinion, there is no doubt that a cheap bevacizumab and an expensive ranibizu-

mab are both equally well for patients suffering from vision-threatening neovascular AMD, yet the option of a cheaper drug seems to be a good choice.

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Neurobiology and pharmacology of fear

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Fear is an important adaptive reaction serving to predict danger and avoid harmful experiences. Fear responses can be divided into the two main categories: inborn reactions inherited from our ancestors during phylogeny of a humankind (e.g. fear of spiders), and responses acquired in individual life during Pavlovian fear conditioning where an emotionally neutral conditioned stimulus is paired with an aversive unconditioned stimulus, for example a foot shock. The second category of fear responses is closely interrelated with inborn fear reactions by using previously developed and hard-wired into the brain mechanisms of fear stimuli processing and behavioral expression. Thus, the neural mechanisms that are subserving these functions are evolutionarily old, and their dysfunction is thought to underlie of anxiety disorders in humans, including post-traumatic stress and panic disorder. The brain circuitry controlling fear responses involves limbic structures, with the amygdala, hippocampus, hypothalamus and periaqueductal gray, playing the fundamental role. All of these structures are under inhibitory control of frontal cortex, and particularly the prefrontal cortex. The basolateral amygdala nuclei are particularly important not only for the "production" and the output of fear responses, but also for the process of an active fear extinction. Recently, the existence of both fear and extinction neurons has been inferred from changes in the activity of basolateral amygdala after learning of fear and extinction. The brain structures, including the prefrontal cortex and limbic system, are densely innervated by monoaminergic, amino acids and peptidergic neurons (NA, 5-HT, GABA, CRF, glutamate). Some of these neurotransmitter systems are the targets for a well recognized anxiolytic drugs, including benzodiazepines and buspirone. However, it should be stressed that their effects are not selective and are secondary to the general inhibitory influence on diverse brain functions. The anxiolytic effects can be induced by many other drugs and psychoactive substances, e.g. barbiturates, alcohol, first generation antihistaminergic drugs, depending on a dose. From this point of view, an anxiolytic effect can be viewed as a stage of their general depressant properties, extending from mild sedation to coma. Accordingly, the most widely used anxiolytic drugs – benzodiazepines are also used as effective hypnotics. Furthermore, it would be unwise to assume that only a single neurotransmitter or receptor system is selectively and exclusively involved in the control of such complex behavioral patterns as fear responses. Similar situation concerns the pathogenesis of other mental disorders, and the possibility of a development of the new selective antipsychotic and antidepressant drugs. Nevertheless, there are still in progress many efforts to develop new selective anxiolytic drugs, including CRF receptor antagonists and mGluRs ligands. Being pessimistic as to the final success of such efforts I deeply believe that these studies can help to considerably extent our knowledge on the pathomechanism of affective disorders, including anxiety.

Brain cytochromes P-450

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CYP2D6 is an enzyme involved in the metabolism of many drugs active in the central nervous system, such as antipsychotics, antidepressants, and central opioids. CYP2D6 is coded by a polymorphic gene. Beside its expression in the liver, CYP2D6 is widely expressed in the brain, but its function there is not well understood. In man, *CYP2D6* mRNA and protein have been shown to be expressed in neurons, with preferential localization in the hypothalamus, hippocampal cortex, substantia nigra, cerebellum, and neocortex.

There are large numbers of speculations on the role of CYPs in the brain. CYP2D6 has been shown to play a role in the biotransformation of precursors to endogenous transmitters such as dopamine and serotonin. Recently, it has also been shown that CYP2D6 may play a role in morphine biosynthesis. Some studies have attempted to establish an association between a personality phenotype and *CYP2D6* genotype.

In recent years, there is increasing evidence that P450-mediated metabolism of psychoactive drugs directly in the brain can lead to local pharmacological modulation at the site of action and result in variable drug response. The inter-individual variability in hepatic metabolism of drugs caused by genetic polymorphism exhibited by some forms of P450, such as P4502D6, is reflected in the plasma levels of administered drugs. But plasma drug levels often show poor correlation with therapeutic effect suggesting that metabolism within the brain could influence the therapeutic outcome regardless of hepatic clearance and plasma drug levels. A moderate difference in the pharmacokinetics of psychoactive drugs often leads to dramatic pharmacodynamic effects suggesting that metabolism *in situ* within the brain could play a significant role.